

Structural Premise of Selective Deubiquitinase USP30 Inhibition by Small-Molecule Benzosulfonamides

Darragh P O'Brien^{1*}, Hannah BL Jones¹, Franziska Guenther², Emma J Murphy², Katherine S England², Iolanda Vendrell¹, Malcolm Anderson³, Paul Brennan², John B Davis², Adán Pinto-Fernández^{1,4}, Andrew P Turnbull⁵, and Benedikt M Kessler^{1,4*}

¹Target Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, UK

²ARUK-Oxford Drug Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, UK

³Waters Corporation, Wilmslow, Cheshire, UK

⁴Chinese Academy of Medical Sciences Oxford Institute, Nuffield Department of Medicine, University of Oxford, UK

⁵Cancer Research Horizons, Francis Crick Institute, London, UK

*Corresponding authors: darragh.obrien@ndm.ox.ac.uk; benedikt.kessler@ndm.ox.ac.uk

SUPPLEMENTAL FIGURES

Contents

Figure S1: Purity profile of USP30_{inh}

Figure S2: Confirmation of USP30 complex formation with USP30_{inh}

Figure S3: Inhibition of USP30 cleavage of K6-linked Di-Ubiquitin by USP30_{inh} as measured by RapidFire MS

Figure S4: Peptide map of USP30

Figure S5: HDX-MS uptake curves for individual USP30 peptides

Figure S6: Differential HDX-MS of USP30 and USP30_{inh}

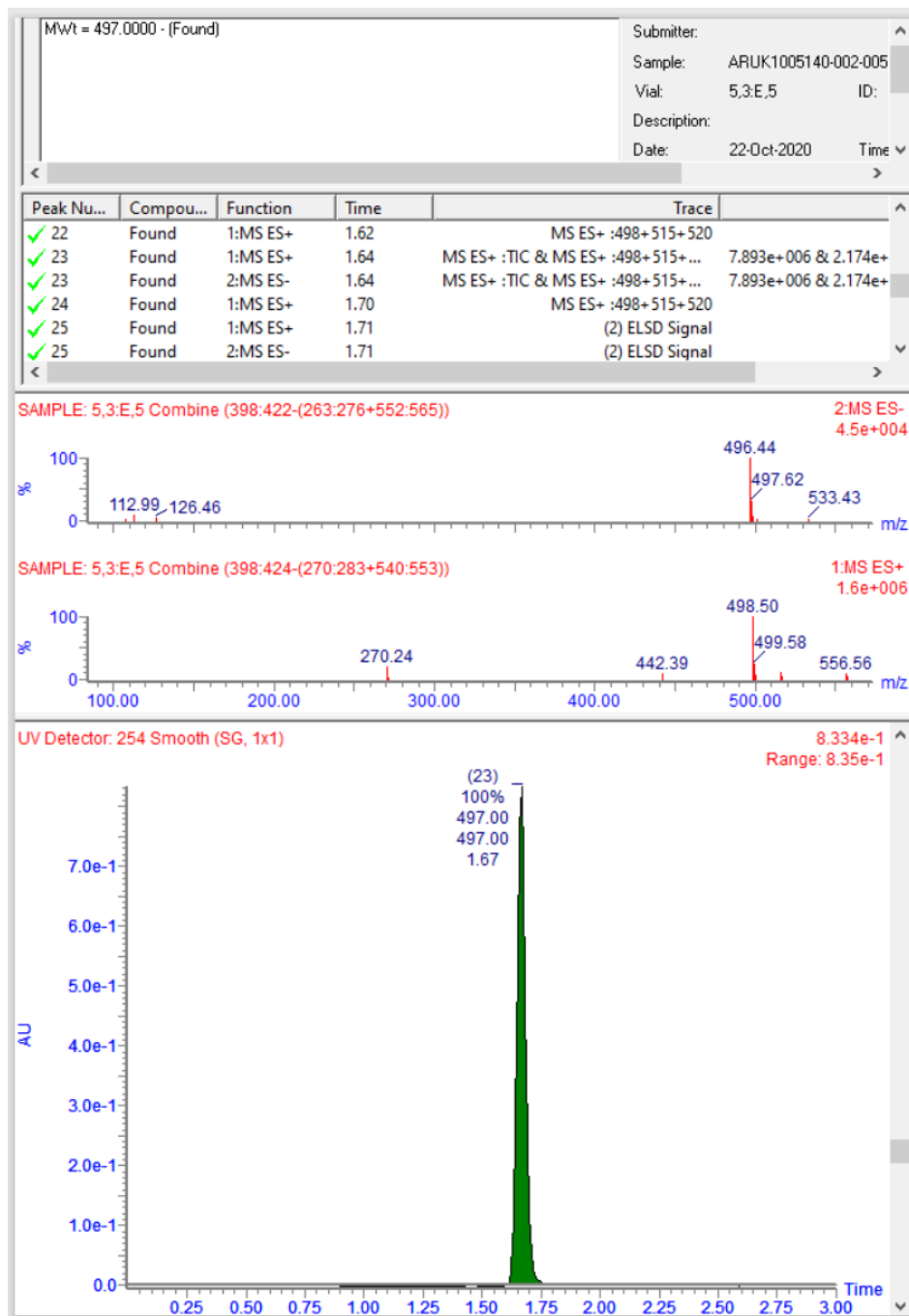
Figure S7: Architecture of USP30 inferred by HDX-MS

Figure S8: Temporal exchange of USP30

Figure S9: Structure of human USP30 in complex with the covalent inhibitor, 552

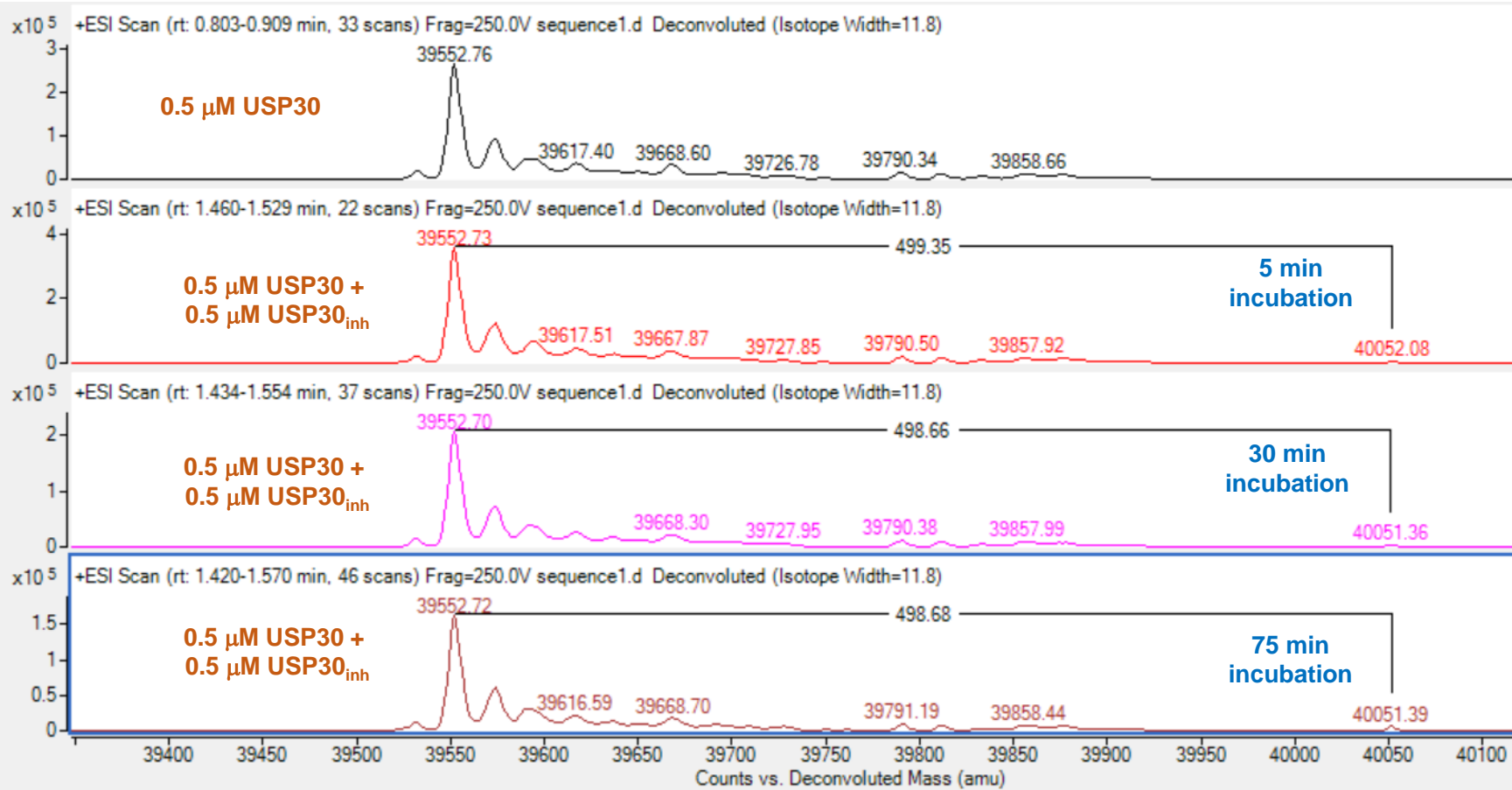
Figure S10: Structure of human USP30 in complex with the covalent inhibitor, 829

Supplemental Figure 1



Supplemental Figure 2

1:1 USP30:USP30_{inh}



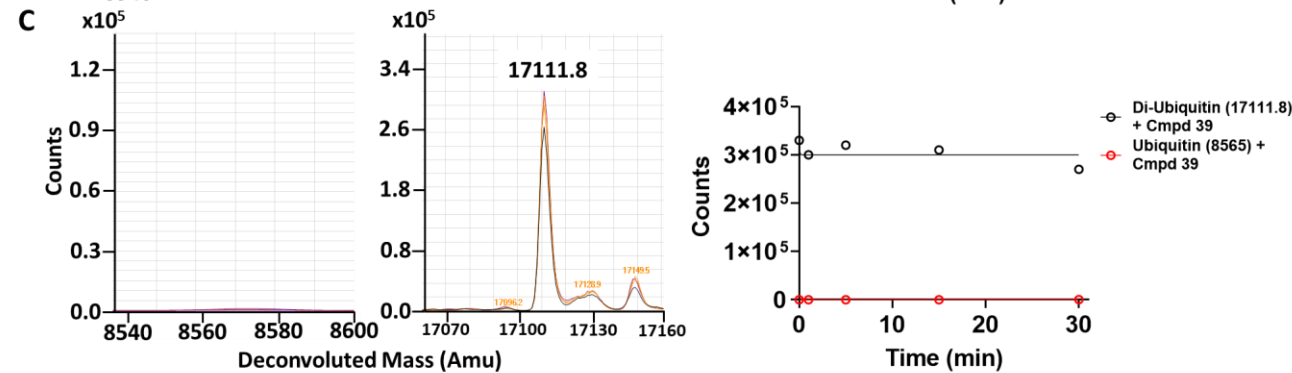
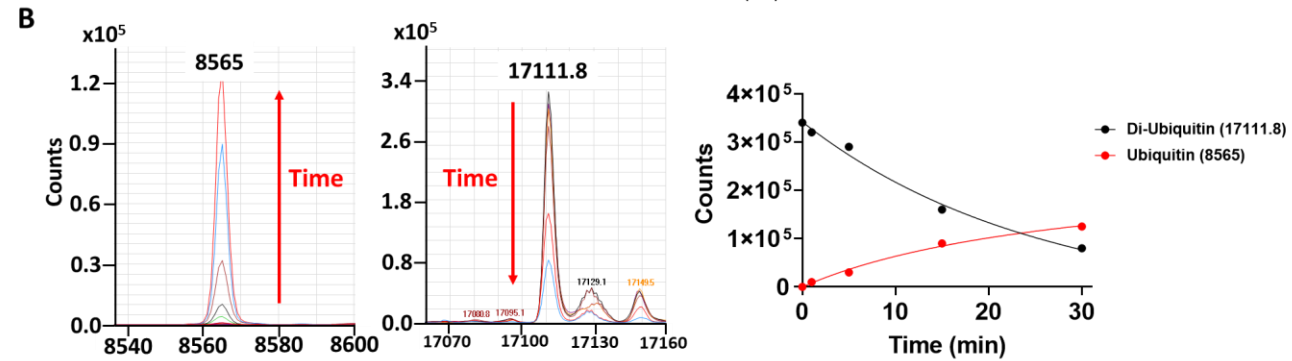
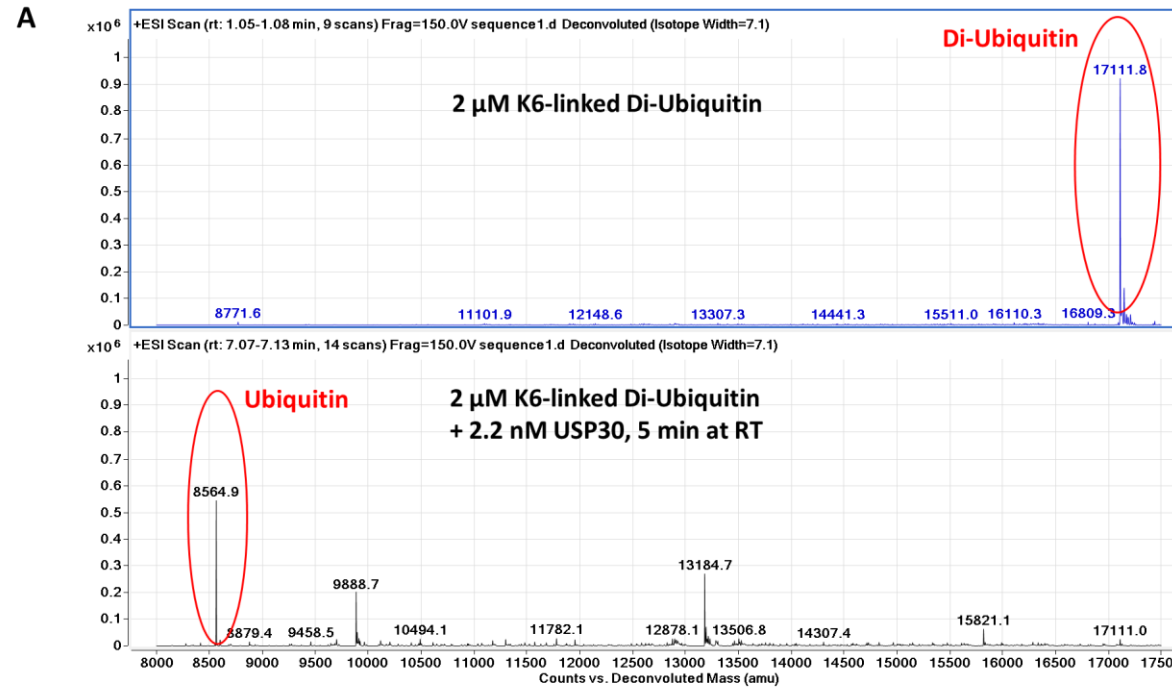
USP30_{inh}

MW: 497.6

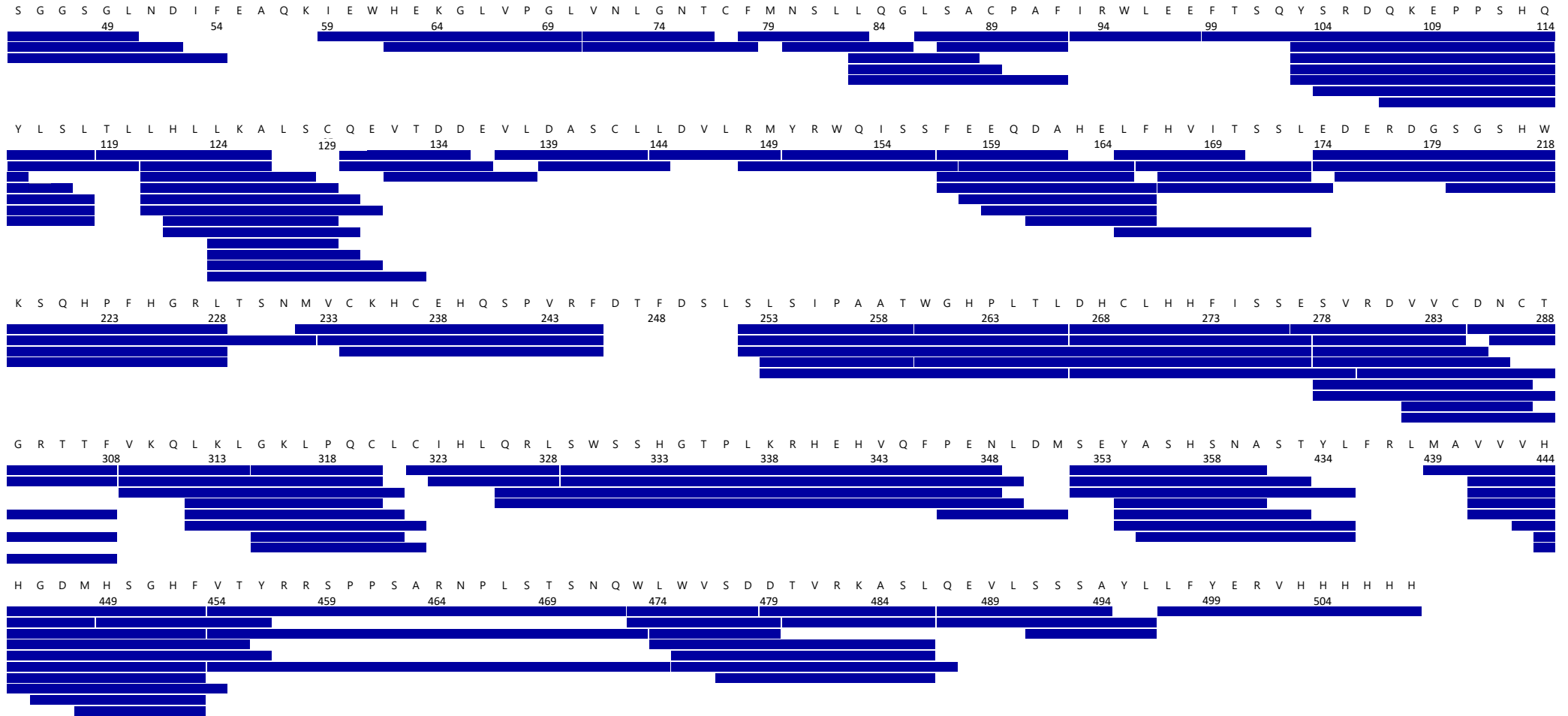
USP30 (Viva)

- **MW: 39552.24 Da**
- insertion/deletion mutant: (64-178)-GSGS-(217-288)-G-(305-357) N346P, F348N, M350D, D352S, I353E, K355A, Y356S-SNA-(432-502)-6*His + biotin tag

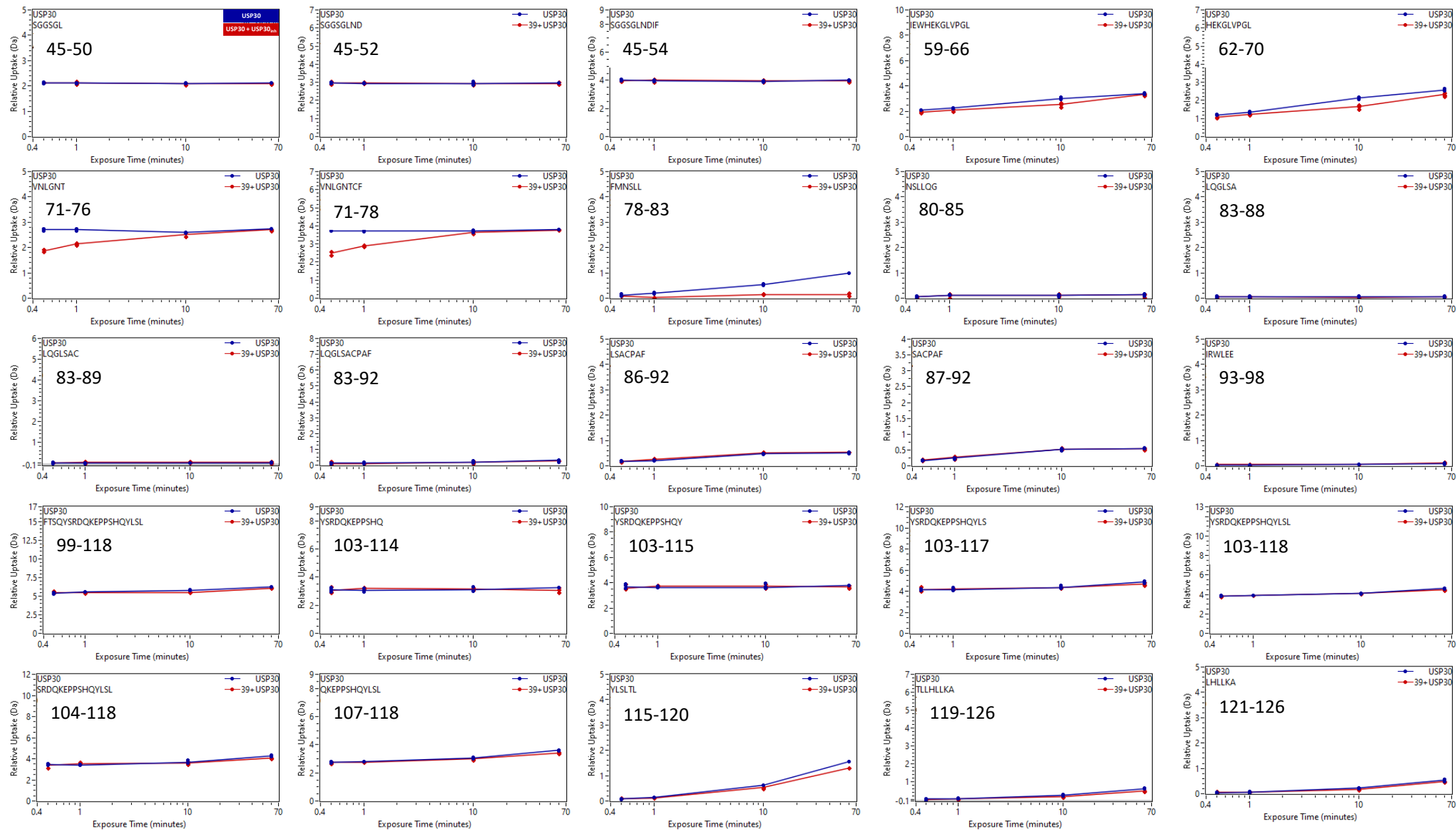
Supplemental Figure 3



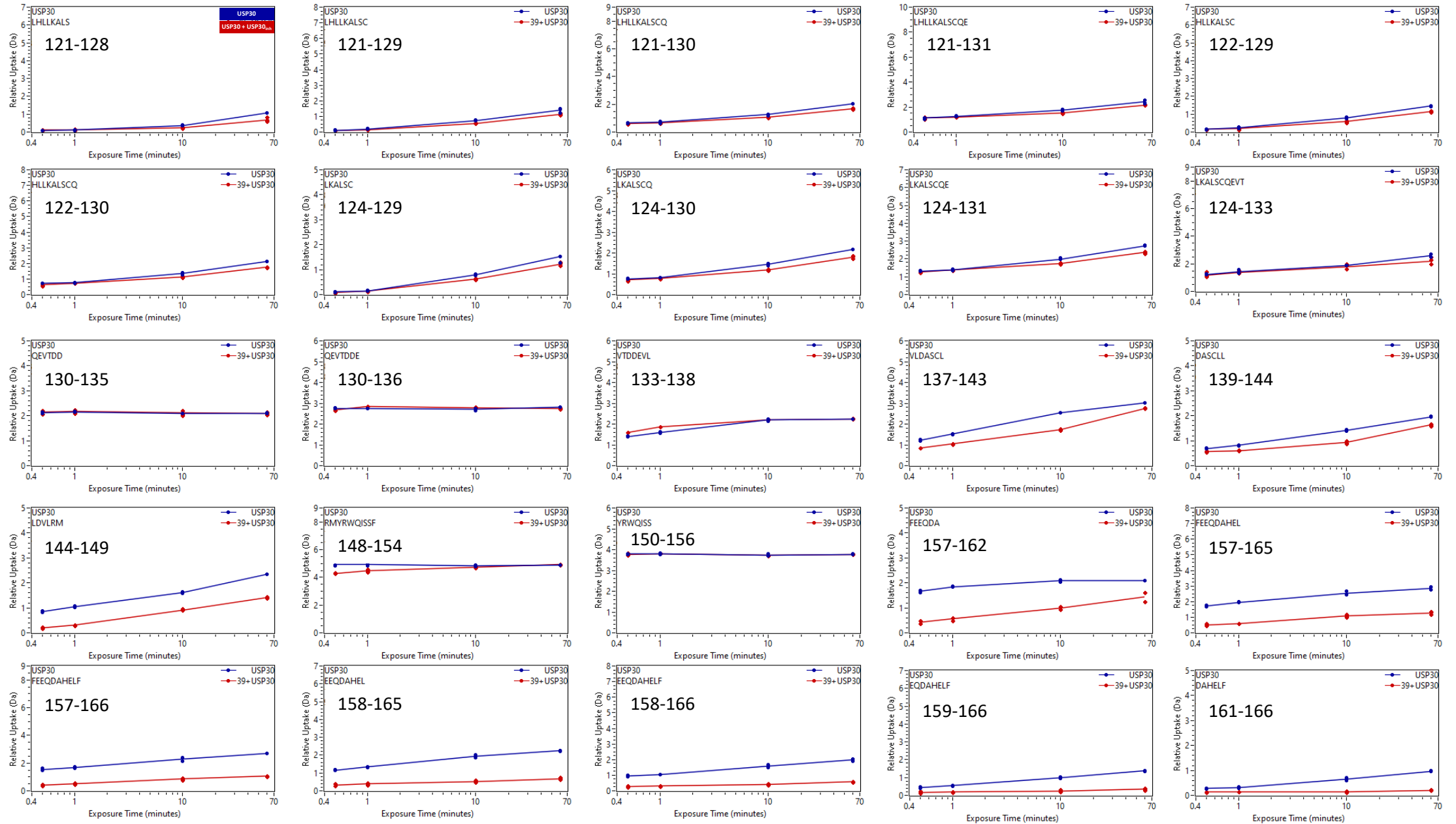
Supplemental Figure 4



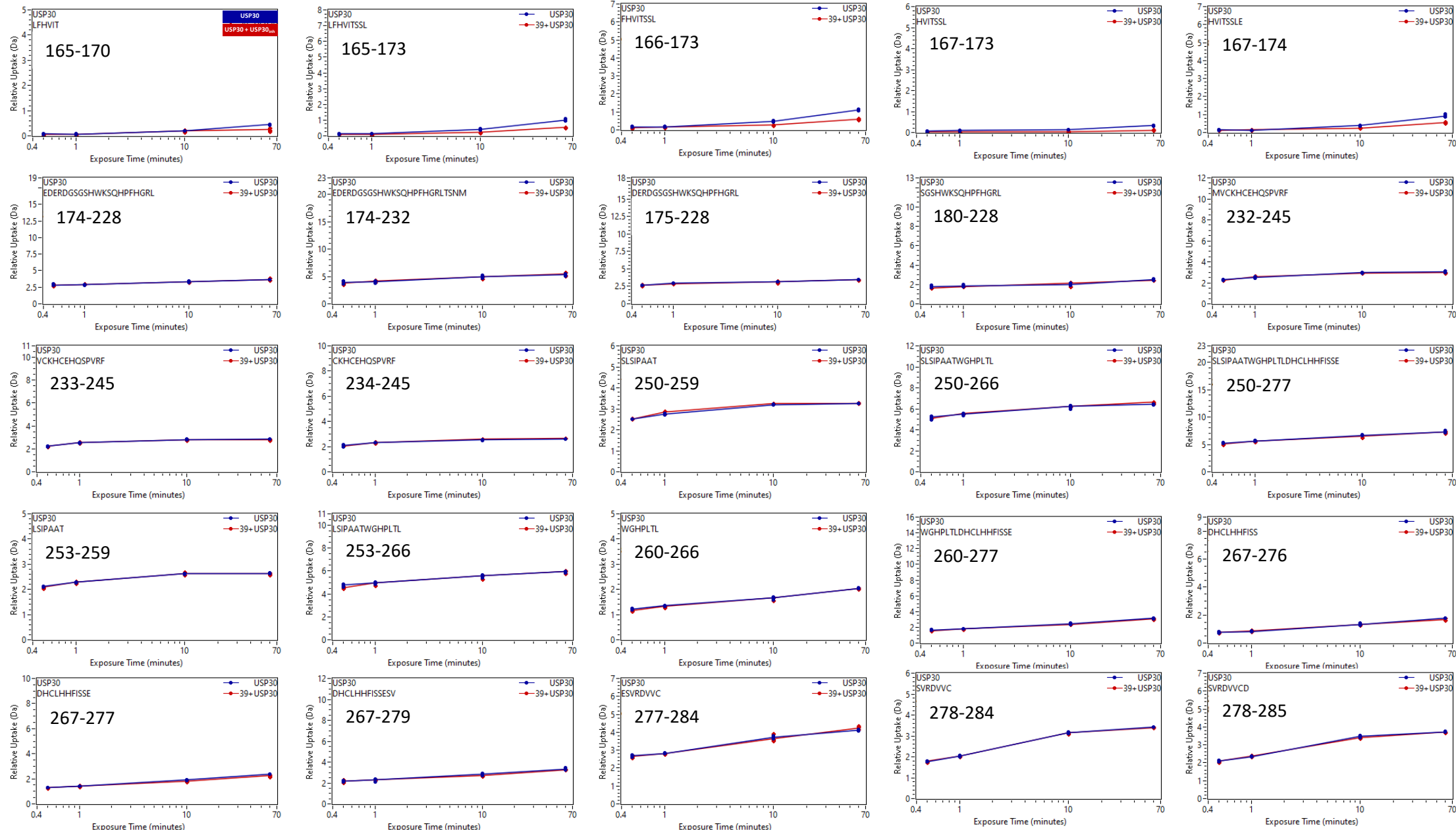
Supplemental Figure 5



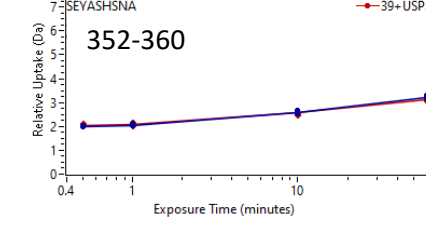
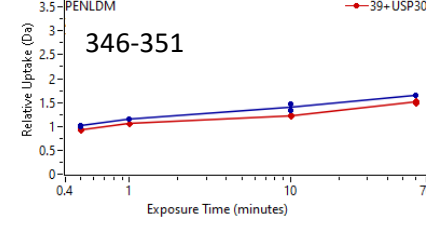
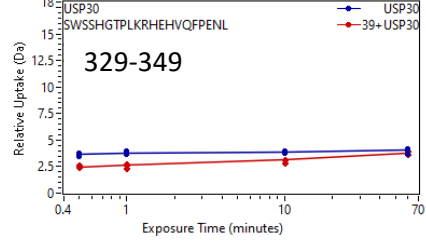
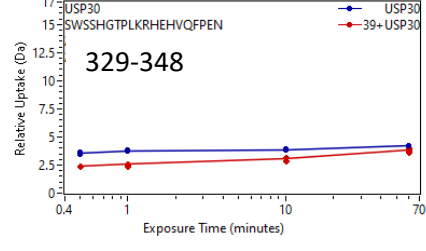
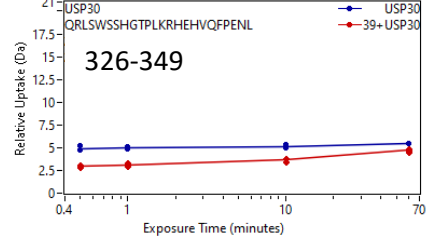
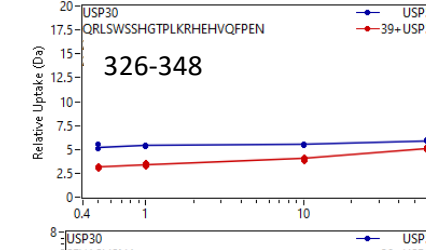
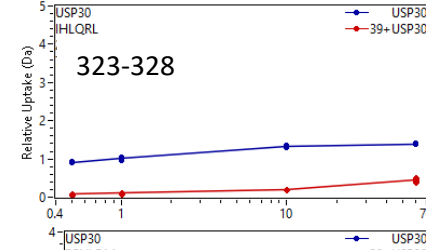
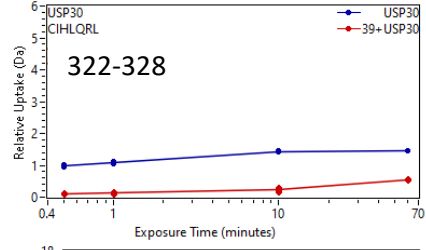
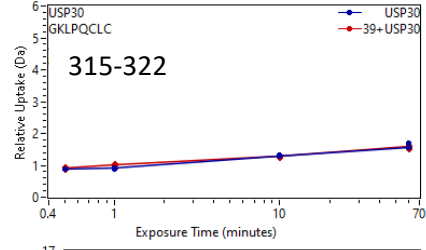
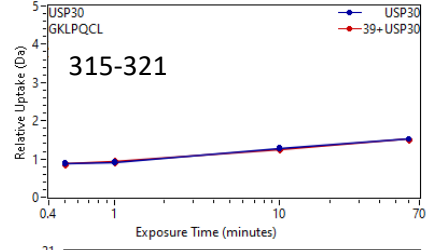
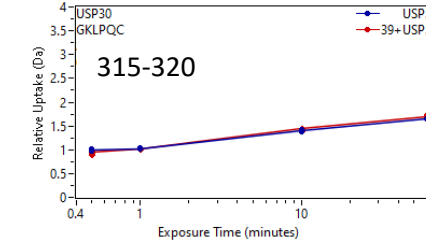
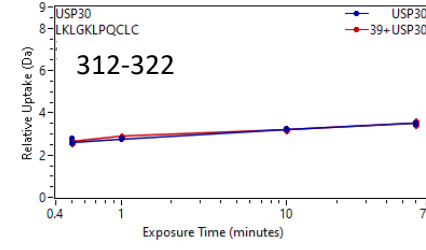
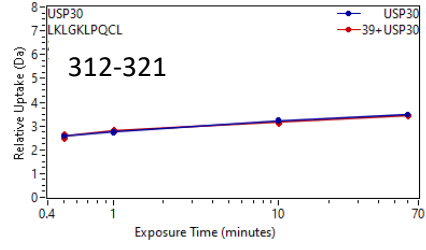
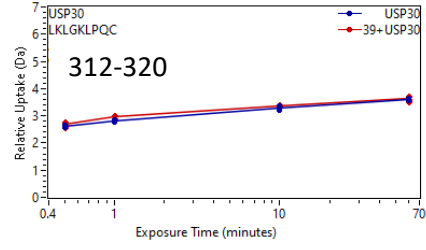
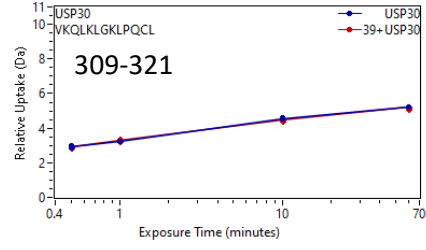
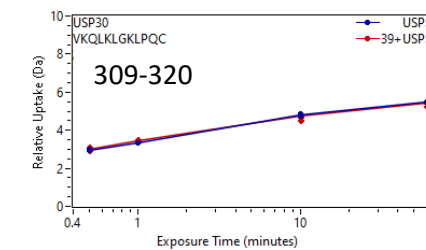
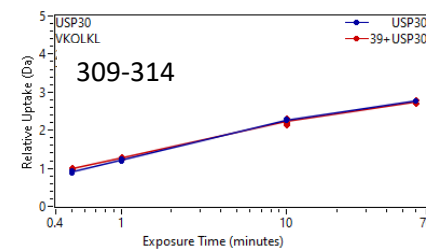
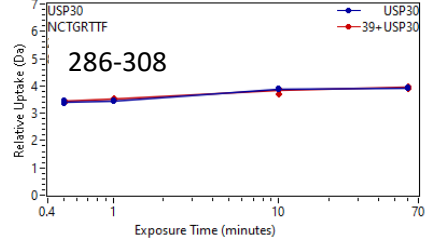
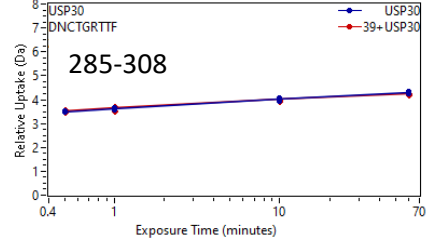
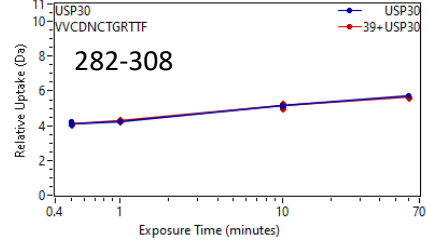
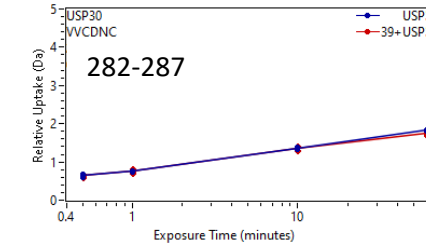
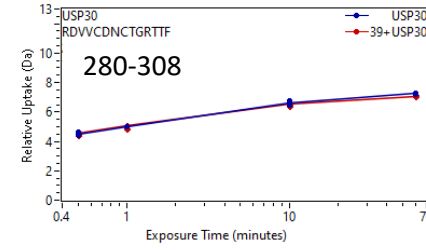
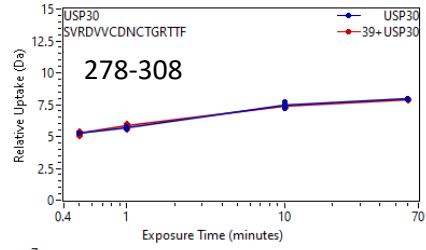
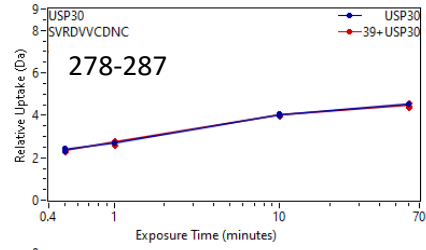
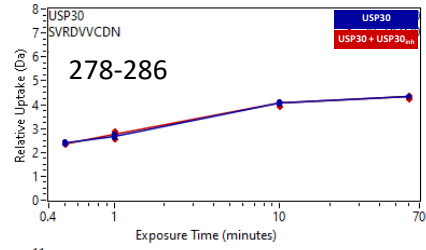
Supplemental Figure 5 (continued)



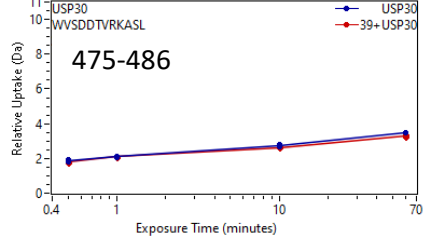
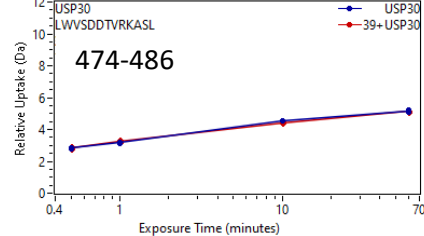
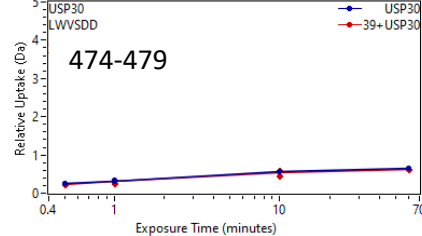
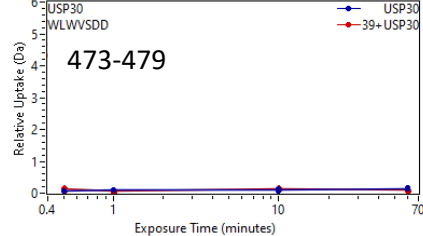
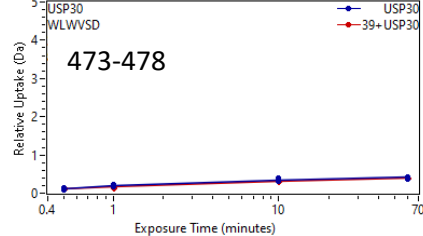
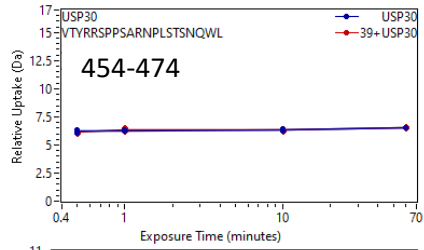
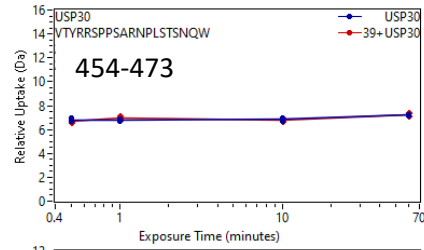
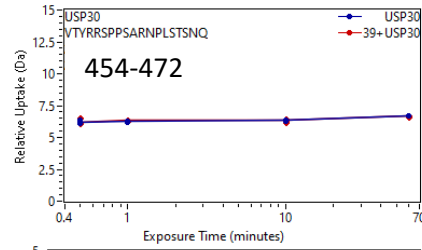
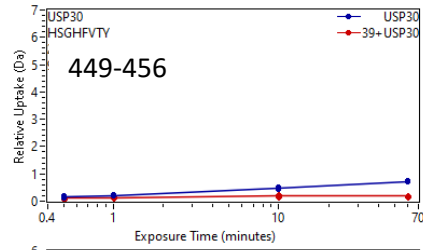
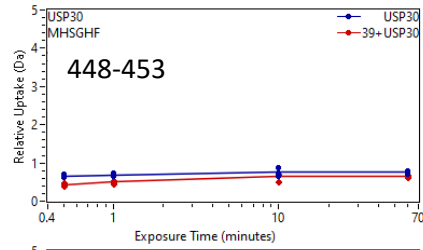
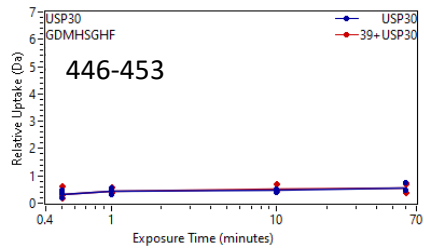
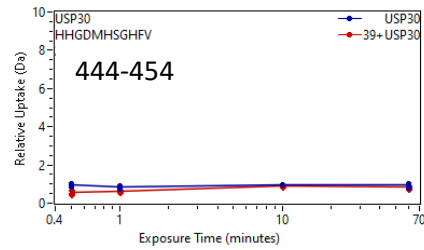
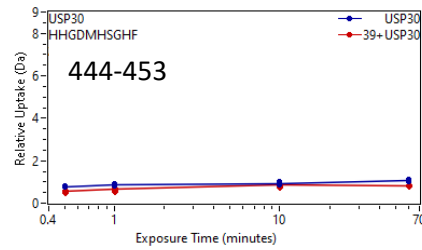
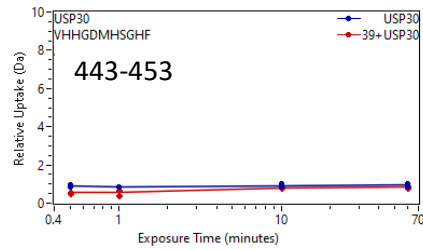
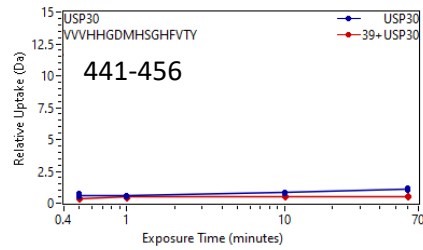
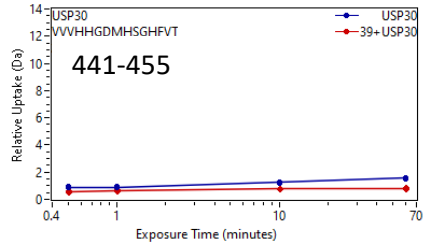
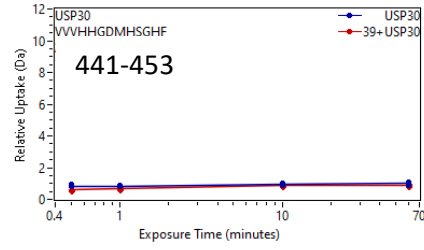
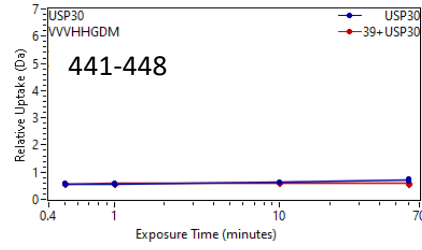
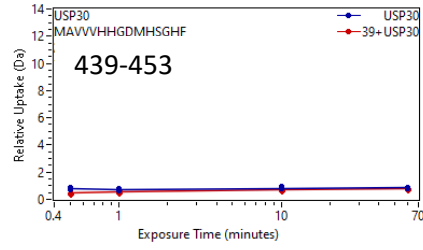
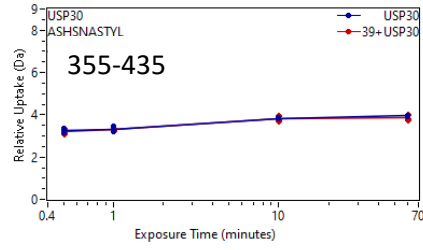
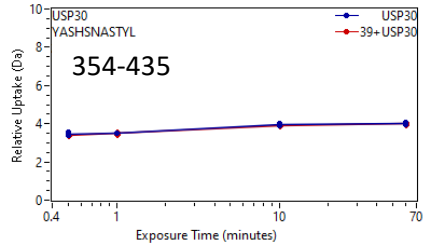
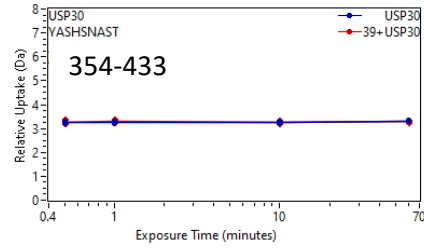
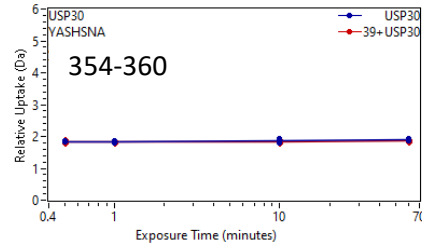
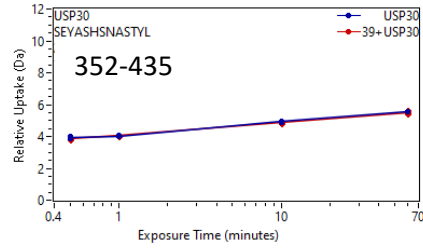
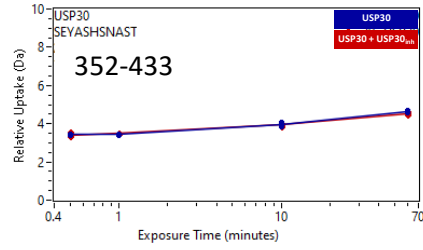
Supplemental Figure 5 (continued)



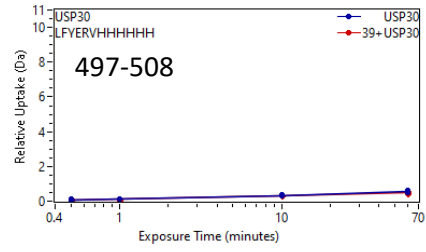
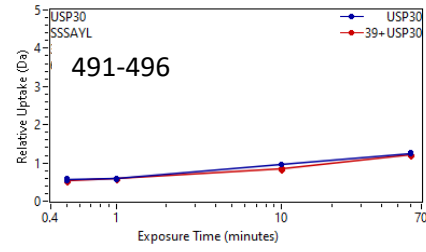
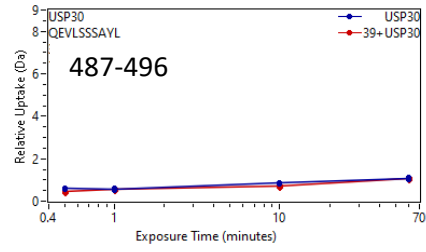
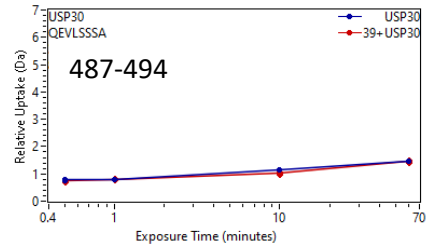
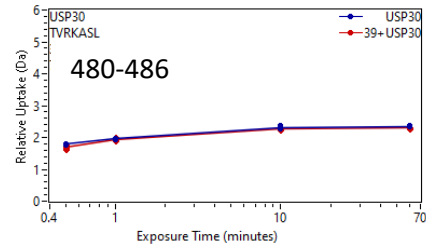
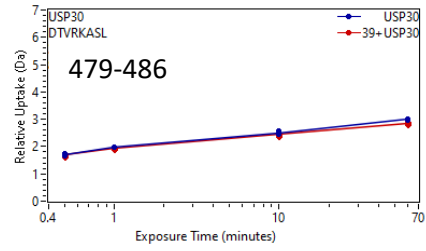
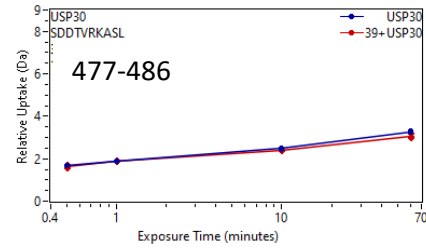
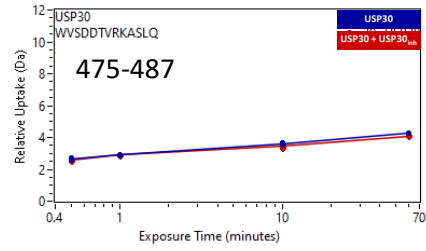
Supplemental Figure 5 (continued)



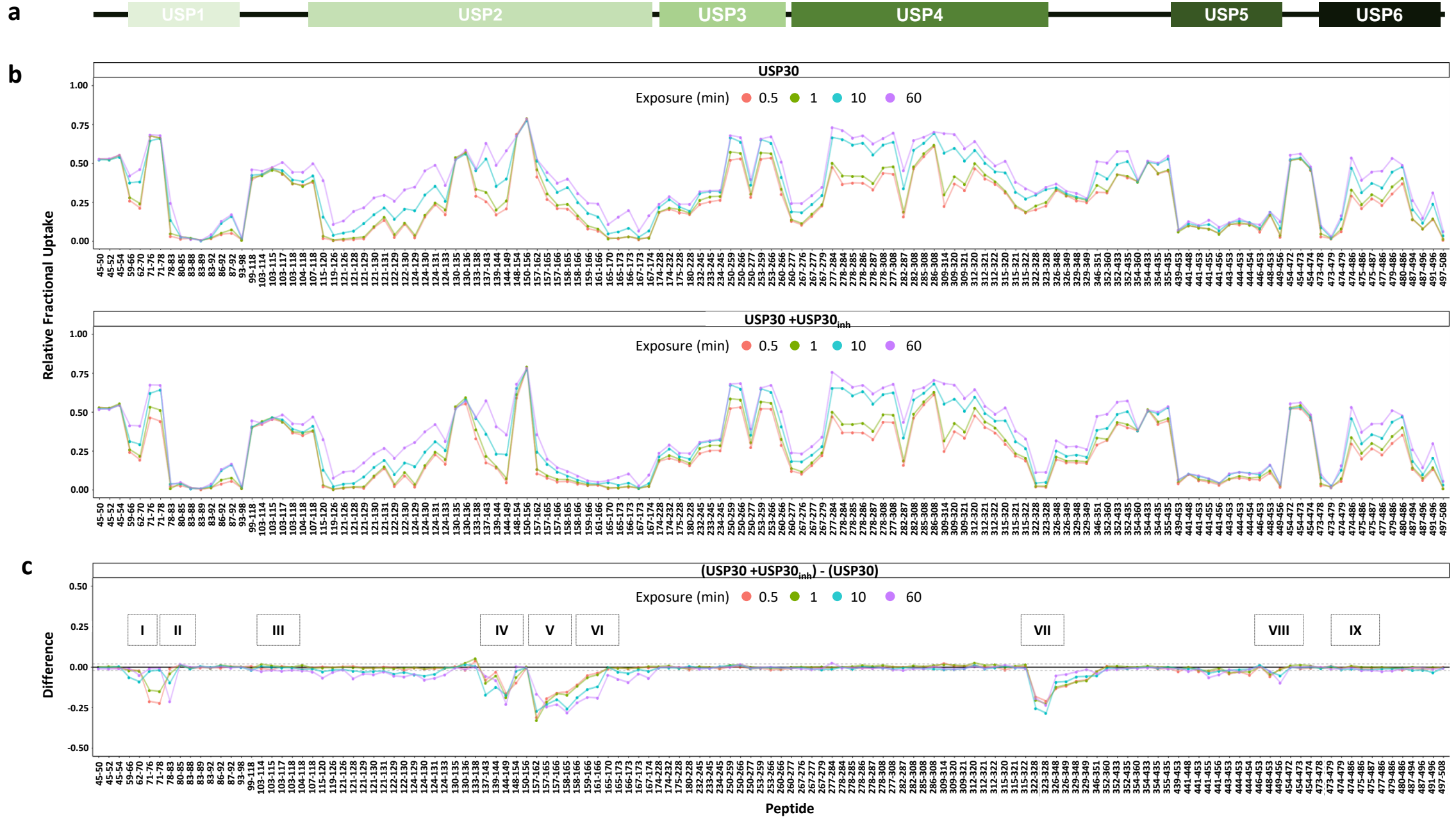
Supplemental Figure 5 (continued)



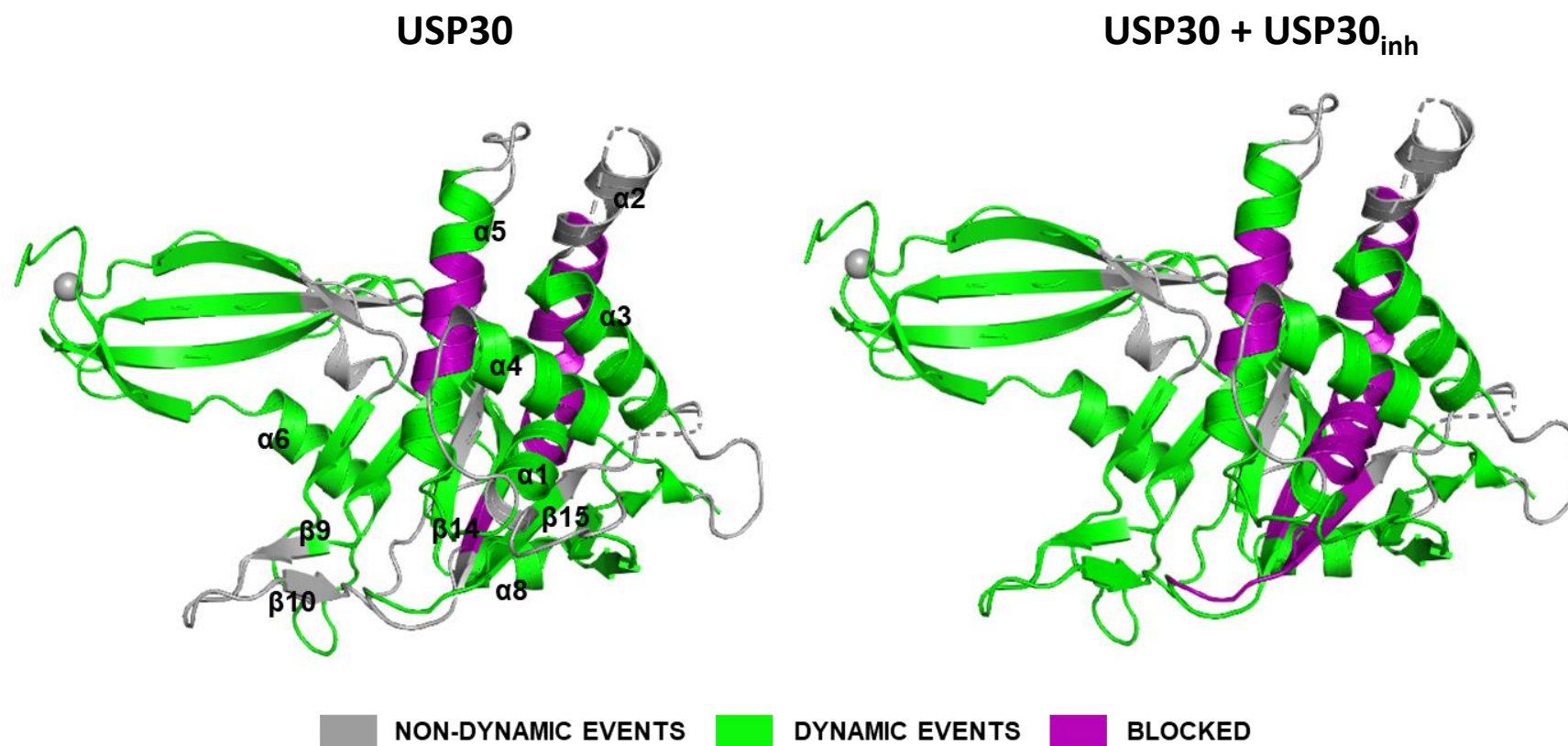
Supplemental Figure 5 (continued)



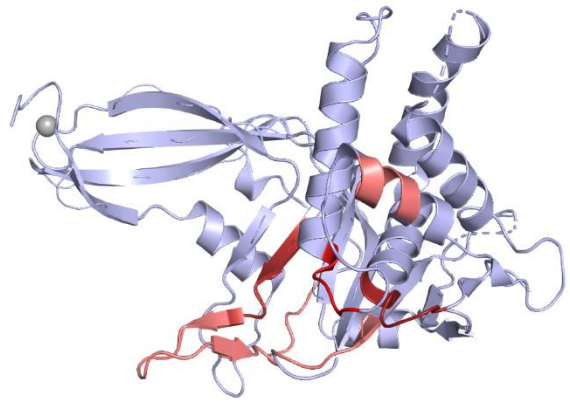
Supplemental Figure 6



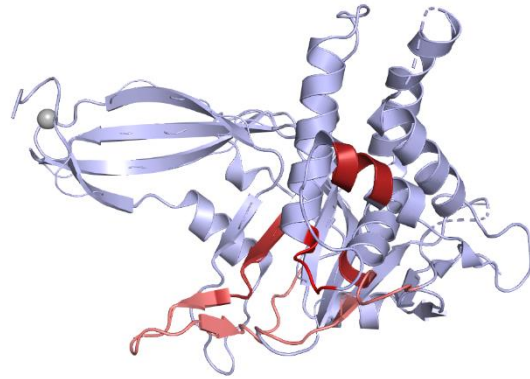
Supplemental Figure 7



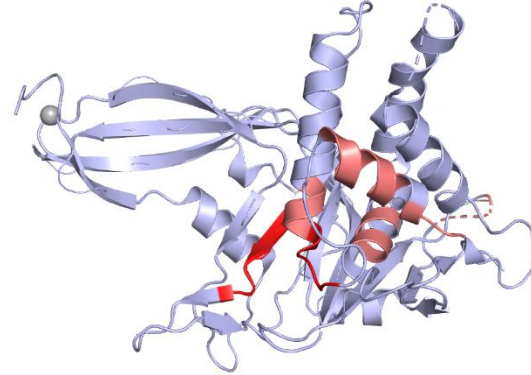
Supplemental Figure 8



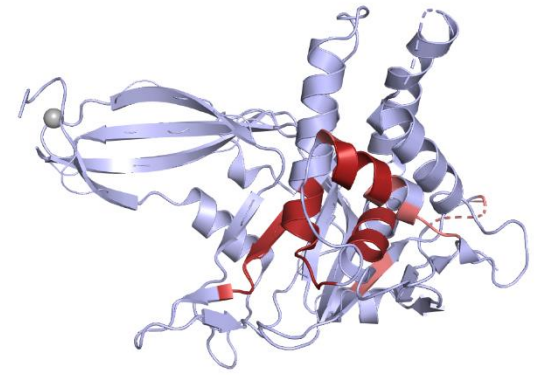
30 sec



60 sec



600 sec

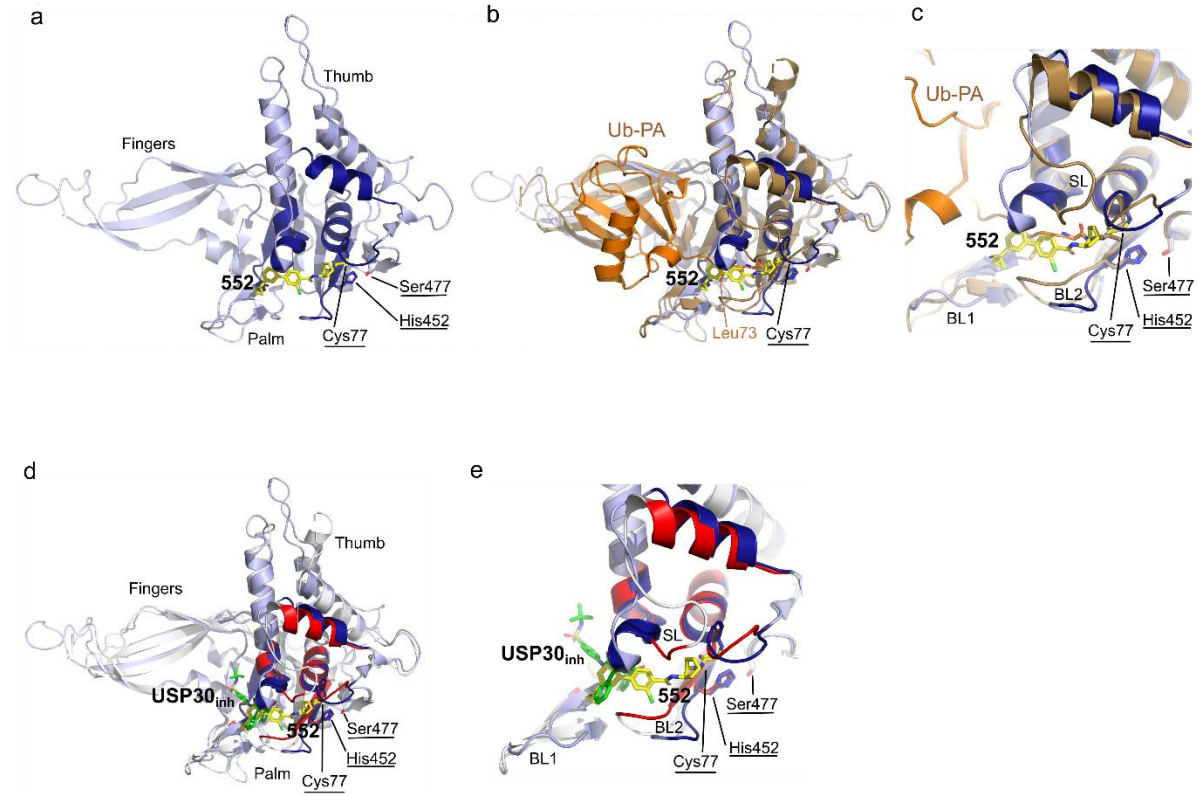


3600 sec

PERTURBATION



Supplemental Figure 9



Supplemental Figure 10

